Miltenyi Biotec

CD4+ Dendritic Cell Isolation Kit
mouse

Order no. 130-091-262

Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling of T, B, NK cells, and granulocytes
   - 2.3 Magnetic separation: Depletion of T, B, NK cells, and granulocytes
   - 2.4 Magnetic labeling of CD4+ dendritic cells
   - 2.5 Magnetic separation: Positive selection of CD4+ dendritic cells
3. Example of a separation using the CD4+ Dendritic Cell Isolation Kit
4. References

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components

1 mL CD4+ Dendritic Cell Biotin-Antibody Cocktail, mouse:
Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD90, CD45R, CD49b, CD8a, CD3, and Ly-6G.

2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).

2 mL CD4 (L3T4) MicroBeads:
MicroBeads conjugated to monoclonal anti-mouse CD4 antibody (isotype: rat IgG2a).

Capacity
For 2×10^9 total cells.

Product format
CD4+ Dendritic Cell Biotin-Antibody Cocktail, Anti-Biotin MicroBeads, and CD4 (L3T4) MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage
Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

The isolation of CD4+ mouse dendritic cells is performed in a two-step procedure. First, T, B, NK cells, and granulocytes are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, CD4+ dendritic cells are directly labeled with CD4 (L3T4) MicroBeads and isolated by positive selection from the pre-enriched dendritic cell fraction. The magnetically labeled CD4+ dendritic cells are retained within the column and eluted after removing the column from the magnetic field. To increase the purity, the positively selected cell fraction containing the CD4+ dendritic cells is separated over a second column.

Single cell suspension: Depletion of T, B, NK cells, and granuloscytes

1. Indirect magnetic labeling of T, B, NK cells and granulocytes with Biotin-Antibody Cocktail and Anti-Biotin MicroBeads.
2. Magnetic separation using an LD Column or an autoMACS Column (program "Depl025").

Pre-enriched dendritic cells (flow-through fraction): Positive selection of CD4+ dendritic cells

1. Direct magnetic labeling of CD4+ dendritic cells with CD4 (L3T4) MicroBeads.
2. Magnetic separation using an MS Column or an autoMACS Column (program "Posseld2").

CD4+ dendritic cells (eluted fraction)

1.2 Background information

Dendritic cells are a rare, heterogeneous population of hematopoietic cells. This kit has been designed for the isolation of CD4+ dendritic cells, which constitute one of the three major dendritic cell subpopulations in mouse spleen.[1] CD4+ dendritic cells express CD11c, CD11b, MHC class II, CD40, CD80, and CD86, and are negative for CD8 and CD205. CD4+ dendritic cells represent the major population of dendritic cells in spleen and comprise only a small population in peripheral lymph nodes (56% and 4% of all CD11c+ dendritic cells, respectively).[2,3] They are mainly located in the marginal zone between the red and white pulp and migrate into the T cell zones upon stimulation with microbial stimuli.[4]

1.3 Applications

- Isolation of CD4+ dendritic cells from single-cell suspensions of spleen for the analysis of antigen-uptake and antigen processing, T cell activation or T cell tolerance induction, cross-priming of cytotoxic T cells, T cell polarization by CD4+ dendritic cells.

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1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of T, B, NK cells, and granulocytes is performed on an LD Column. The subsequent positive selection of $CD4^+$ dendritic cells is performed on two MS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the SuperMACS™ Separator. For details see the respective MACS Separator data sheet.

- Collagenase D: 2 mg/mL (Collagenase D >0.15 U/mg, e.g., from Roche Diagnostics, Germany) in 10 mM Hepes-NaOH, pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD11c Antibody, anti-human, FITC, CD11c Antibody, anti-human, PE or CD11c Antibody, anti-human, APC. For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

  ▲ **Note:** Staining of isolated cells with CD4 has to be performed with a clone different from GK1.5.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

▲ For highest recovery and purity of $CD4^+$ dendritic cells from mouse spleen, single-cell suspensions have to be prepared by enzymatic disaggregation with Collagenase D. Protocols which rely only on mechanical disruption are not recommended.

1. Place isolated spleen in a 6 cm petri-dish with sufficient Collagenase D solution to completely cover the bottom of the dish (5 mL/spleen).
2. Inject mouse spleen with 500 µL of Collagenase D solution per spleen using a 1 mL syringe and a 25G needle, then cut the tissue into smaller pieces using sharp scissors.
3. Incubate the spleen pieces in Collagenase D solution for 30 minutes at 37 °C.
4. Pass the whole material, i.e. remaining fragments and Collagenase D-released cells, gently through a 70 µm cell strainer using a plunger.
5. Collect all cells in a 50 mL tube and wash the cells by adding buffer to a final volume of 15 mL.

   ▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells, removal of dead cells by density gradient centrifugation or by using the Dead Cell Removal Kit (# 130-090-101) is recommended.

6. Proceed to magnetic labeling (2.2).



2.2 Magnetic labeling of T, B, NK cells and granulocytes

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^8 total cells. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 200 µL of buffer per 10^8 cells.
4. Add 50 µL of Biotin-Antibody Cocktail per 10^8 cells.
5. Mix well and incubate for 10 minutes on ice.
6. Add 150 µL of buffer and 100 µL of Anti-Biotin MicroBeads per 10^8 cells.
7. Mix well and incubate for 15 minutes on ice.
8. Wash cells by adding 10–20× the labeling volume of buffer and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.

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9. Resuspend cell pellet in 500 µL of buffer:
   Depletion with LD Column: 500 µL for up to 1.25×10^8 cells.
   Depletion with autoMACS Pro Separator:
   500 µL for up to 1×10^8 cells.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of T, B, NK cells, and granulocytes

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled pre-enriched dendritic cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the isolation of $CD4^+$ dendritic cells.

Depletion with the autoMACS Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

Depletion with the autoMACS Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. Choose the following program:
   Depletion: "Depl025"
   Collect negative fraction in row B of the tube rack.
4. Proceed to magnetic labeling of $CD4^+$ dendritic cells (2.4).



2.4 Magnetic labeling of $CD4^+$ dendritic cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 total cells. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer.
3. Add 100 µL of CD4 (L3T4) MicroBeads.
4. Mix well and incubate for 15 minutes on ice.
5. Wash cells by adding 10–20× the labeling volume of buffer and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of $CD4^+$ dendritic cells

Positive selection with MS Columns

▲ To achieve highest purities, perform two consecutive column runs.

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase the purity of $CD4^+$ dendritic cells, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection with the autoMACS Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

Positive selection with the autoMACS Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. Choose the following program:
   Positive selection: "Posseld2"
   Collect positive fraction in row C of the tube rack.

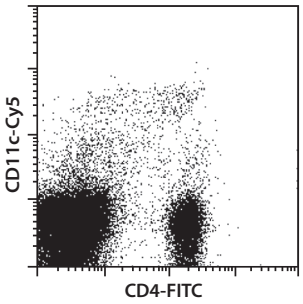
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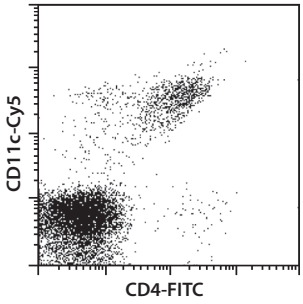
3. Example of a separation using the CD4$^+$ Dendritic Cell Isolation Kit

CD4$^+$ dendritic cells were isolated from mouse spleen cell suspension using the CD4$^+$ Dendritic Cell Isolation Kit, an LD and two MS Columns, a MidiMACS Separator, and a MiniMACS Separator. Cells are fluorescently stained with CD11c-Cy5 and CD4-FITC. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

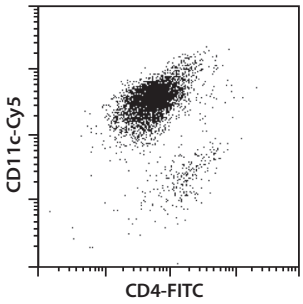
Spleen cells before separation



Pre-enriched CD11c$^+$ dendritic cells after depletion of T, B, NK cells, and granulocytes



Isolated CD4$^+$CD11c$^+$ dendritic cells



4. References

1. Shortman, K. and Liu, Y. J. (2002) Mouse and human dendritic cell subtypes. Nat. Rev. Immunol. 2: 151–161.
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4. De Smedt, T. *et al.* (1996) Regulation of dendritic cell numbers and maturation by lipopolisaccarides in vivo. J. Exp. Med. 184: 1413–1424.

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